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Inventors: Breslauer et al.
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application:

Abstract

Simple methods and kits for determining the thermodynamic stability of nucleic acid duplexes and single polynucleotide polymorphisms via competitive equilibria are provided.

In the Claims:

Please amend the claims as follows:

1. (amended) A method for screening for nucleic acid duplex stability by competitive equilibria comprising:

(a) producing a solution containing a known amount of an initial nucleic acid duplex with a known stability, said initial nucleic acid duplex comprising a first nucleic acid strand having a sequence wholly or in part homologous to a target strand and a second nucleic acid strand having a sequence wholly or in part complementary to the target strand;

(b) titrating the solution with a second solution comprising a known concentration of the target nucleic acid strand which competes with the first nucleic acid strand of the initial nucleic acid duplex of step (a) for binding to the second nucleic acid strand of the initial nucleic acid duplex of step (a), said

target nucleic acid strand being single- or double-stranded;

(c) subjecting the titrated solution to conditions which disrupt the initial nucleic acid duplex of step (a) and any duplex or triplex formed between the target strand and the second nucleic acid strand of the initial nucleic acid duplex of step (a) upon titration in step (b), but which do not disrupt the target strand when double-stranded;

(d) subjecting the titrated solution to conditions which promote duplex or triplex formation; and

(e) monitoring the titrated solution for changes in the amount of initial nucleic acid duplex formed as a function of the amount of target nucleic acid strand added.

2. (amended) The method of claim 1 wherein the conditions in step (c) comprise heating the titrated solution to a temperature high enough to disrupt the initial nucleic acid duplex of step (a) and any duplex or triplex formed between the target strand and the second nucleic acid strand of the initial nucleic acid duplex of step (a) upon titration in step (b), but which do not disrupt the target strand when double-stranded and the conditions of step (d) comprise cooling the titrated solution to a temperature wherein duplex formation occurs.

3. (amended) A method for screening for nucleic acid duplex

stability comprising:

(a) producing a solution containing an initial nucleic acid duplex with a known stability, said initial nucleic acid duplex comprising a first nucleic acid strand and a second nucleic acid strand, each strand being capable of forming a duplex with a double-stranded target strand;

(b) titrating the double-stranded target strand into the solution;

(c) subjecting the titrated solution to conditions which disrupt the initial nucleic acid duplex of step (a), the double-stranded target strand, and any duplex between the disrupted target strands and the first and second nucleic acid strands of the initial nucleic acid duplex of step (a);

(d) subjecting the titrated solution to conditions which promote duplex formation; and

(e) monitoring the titrated solution for changes in the amount of initial nucleic acid duplex formed as a function of the amount of double-stranded target nucleic acid strand added.

4. (amended) The method of claim 3 wherein the conditions in step (c) comprise heating the titrated solution to a temperature high enough to disrupt the initial nucleic acid duplex of step (a), the double-stranded target duplex of step (b)

and any duplexes formed between the disrupted target strands and the first or second nucleic acid strands of the initial nucleic acid duplex of step (a) upon titration in step (b) and the conditions of step (d) comprise cooling the titrated solution to a temperature wherein duplex formation occurs.

5. (amended) A method for extracting enthalpy data from the competitive equilibria method of claim 2 comprising controlling temperature during step (d) so that changes monitored in step (c) can be collected as a function of temperature to produce a family of titration curves that can be used to extract enthalpy (ΔH°) data.

6. (amended) A method for detecting a single nucleotide polymorphism comprising:

(a) producing an initial nucleic acid duplex comprising a first and second nucleic acid strand, wherein the first or second strand of the initial nucleic acid duplex is designed to identify a single nucleotide polymorphism in a single- or double-stranded target nucleic acid sequence;

(b) measuring the amount of the initial nucleic acid duplex produced in step (a);

(c) adding a fixed excess amount of the single- or double-stranded target nucleic acid strand into the solution;

(d) subjecting the solution to conditions which disrupt the initial nucleic acid duplex of step (a) and any duplex or triplex formed between the single- or double-stranded target strand and the first or second nucleic acid strand of the initial nucleic acid duplex of step (a) upon addition of the single- or double-stranded target strand in step (c), but which do not disrupt the target strand when double-stranded;

(e) subjecting the titrated solution to conditions which promote duplex or triplex formation; and

(f) measuring the amount of initial nucleic acid duplex formed after addition of the single- or double-stranded target strand wherein the measured amount after addition of the single- or double-stranded target strand is indicative of the single- or double-stranded target strand containing the single nucleotide polymorphism.

7. (amended) The method of claim 6 wherein the conditions in step (c) comprise heating the titrated solution to a temperature high enough to disrupt the initial nucleic acid duplex of step (a) and any duplex or triplex formed between the single- or double-stranded target strand and the first or second nucleic acid strand of step (a) upon addition of the single- or double-stranded target strand in step (c), but which do not

disrupt the target strand when double-stranded and the conditions of step (d) comprise cooling the titrated solution to a temperature wherein duplex formation occurs.

8. (amended) A method for detecting a single nucleotide polymorphism comprising:

(a) producing an initial nucleic acid duplex comprising a first and second nucleic acid strand, wherein the first or second strand of the duplex is designed to identify a single nucleotide polymorphisms in a double-stranded target nucleic acid sequence;

(b) measuring the amount of the initial nucleic acid duplex;

(c) adding a fixed excess amount of the double-stranded target nucleic acid strand into the solution;

(d) subjecting the solution to conditions which disrupt the initial nucleic acid duplex of step (a), the double-stranded target nucleic acid sequence and any duplex formed between the double-stranded target strand and the first or second nucleic acid strand of the initial nucleic acid duplex of step (a) formed upon addition of the double-stranded target strand in step (c);

(e) subjecting the titrated solution to conditions which promote duplex formation; and

(f) measuring the amount of initial duplex formed after addition of the target strand wherein the measured amount after

addition of the target strand is indicative of the target strand containing the single nucleotide polymorphism.

9. (amended) The method of claim 8 wherein the conditions in step (c) comprise heating the titrated solution to a temperature high enough to disrupt the initial nucleic acid duplex of step (a), the double-stranded target duplex and any duplexes formed between the disrupted target strands and the first or second nucleic acid strands of the initial nucleic acid duplex of step (a) upon addition of the double-stranded target duplex in step (c) and the conditions of step (d) comprise cooling the titrated solution to a temperature wherein duplex formation occurs.

10. (amended) The method of claim 6 wherein one nucleic acid strand of the duplex formed in step (a) contains a sequence corresponding to the targeted single nucleotide polymorphism; and the measured amount of initial duplex formed after addition of the target strand indicative of the target strand containing the single nucleotide polymorphism in step (f) decreases as compared to the amount measured in step (b).

11. (amended) The method of claim 6 wherein one nucleic acid strand of the duplex formed in step (a) is a wild type sequence; and the measured amount of initial duplex formed after

addition of the target strand is indicative of the target strand containing the single nucleotide polymorphism in step (f) is approximately equal to the amount measured in step (b).

12. (amended) A method for determining the concentration of a target nucleic acid sequence comprising:

(a) adding a known volume and concentration of an initial nucleic acid duplex with a known stability to a known volume of a solution containing a target strand, said initial nucleic acid duplex comprising a first nucleic acid strand having a sequence wholly or in part homologous to the target strand and a second nucleic acid strand having a sequence wholly or in part complementary to the target strand;

(b) subjecting the solution to conditions which disrupt the initial nucleic acid duplex of step (a) and any duplex between the target strand and the first nucleic acid strand or the second nucleic acid strand of the initial nucleic acid duplex of step (a);

(c) subjecting the solution to conditions which promote duplex formation; and

(d) determining the relative change in the amount of initial nucleic acid duplex formed in the solution.

13. (amended) A method for determining the concentration of

a target nucleic acid sequence comprising:

(a) adding a known volume of a solution of target strand to a known volume of a solution containing a known concentration of an initial nucleic acid duplex with a known stability, said initial nucleic acid duplex comprising a first nucleic acid strand having a sequence wholly or in part homologous to the target strand and a second nucleic acid strand having a sequence wholly or in part complementary to the target strand;

(b) subjecting the solution to conditions which disrupt the initial nucleic acid duplex and any duplex between the target strand and the first or second nucleic acid strand of the initial nucleic acid duplex;

(c) subjecting the solution to conditions which promote duplex formation; and

(d) determining the relative change in the amount of initial nucleic acid duplex formed in the solution.

14. (amended) The method of claim 12 wherein the conditions in step (c) comprise heating the titrated solution to a temperature high enough to disrupt the initial nucleic acid duplex and any duplex or triplex formed between the target strand and the first or second nucleic acid strand of the initial nucleic acid duplex, but which do not disrupt the target strand

when double-stranded and the conditions of step (d) comprise cooling the titrated solution to a temperature wherein duplex formation occurs.

15. (amended) A method for assessing stability of various selected target strands comprising:

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(a) selecting various target strands;

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(b) performing the method of claim 1 with the same initial nucleic acid duplex and each of the selected target strands; and

(c) comparing monitored changes in the amount of initial nucleic acid duplex formed as a function of the amount of the selected target nucleic acid strand added to ascertain differences in stability of duplexes or triplexes formed by the various target strands.

25. (amended) The method of any of claims 1 through 15 or 38 through 45 wherein at least one nucleic acid strand of the initial duplex comprises an internal loop, a modified base, a modified backbone, or a non-Watson-Crick nucleotide base variation.

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28. (amended) The method of claims 1 through 15 or 38 through 45 wherein at least one nucleic acid strand of the initial nucleic acid duplex is immobilized to a solid support.

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30. (amended) A method for determining the concentration of

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a target nucleic acid sequence comprising:

(a) measuring fluorescence of a known volume of a solution containing a single- or double-stranded target nucleic acid sequence;

(b) adding a known volume and concentration of an initial nucleic acid duplex to the solution, said initial nucleic acid duplex comprising a first nucleic acid strand having a sequence wholly or in part homologous to the target nucleic acid sequence and a second nucleic acid strand having a sequence wholly or in part complementary to the target nucleic acid sequence;

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(c) subjecting the solution to conditions which disrupt the initial nucleic acid duplex and any duplex or triplex formed between the target strand and the first or second nucleic acid strands of the initial nucleic acid duplex, but which do not disrupt the target strand when double-stranded;

(d) subjecting the solution to conditions which promote duplex or triplex formation; and

(e) measuring the fluorescence of the solution after step (d) so that a relative change in the fluorescence can be determined.

31. (amended) A method for determining the concentration of a target nucleic acid sequence comprising:

(a) measuring fluorescence of a solution containing a known volume and concentration of an initial nucleic acid duplex, said initial nucleic acid duplex comprising a first nucleic acid strand having a sequence wholly or in part homologous to the target strand and a second nucleic acid strand having a sequence wholly or in part complementary to the target strand;

(b) adding a known volume of a single- or double-stranded target nucleic acid sequence to the solution;

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(c) subjecting the solution to conditions which disrupt the initial nucleic acid duplex and any duplex or triplex formed between the target strand and the first or second nucleic acid strands of the initial nucleic acid duplex, but which do not disrupt the target strand when double-stranded;

(d) subjecting the solution to conditions which promote duplex or triplex formation; and

(e) measuring the fluorescence of the solution after step (d) so that a relative change in the fluorescence can be determined.

32. (amended) The method of claims 30 or 31 wherein the conditions in step (c) comprise heating the titrated solution to a temperature high enough to disrupt the initial nucleic acid duplex and any duplex or triplex formed between the target strand

and the first or second nucleic acid strand of the initial nucleic acid duplex, but which do not disrupt the target strand when double-stranded and the conditions of step (d) comprise cooling the titrated solution to a temperature wherein duplex formation occurs.

Please add the following new claims:

38. (amended) A method for extracting enthalpy data from the competitive equilibria method of claim 4 comprising controlling temperature during step (d) so that changes monitored in step (e) can be collected as a function of temperature to produce a family of titration curves that can be used to extract enthalpy (ΔH°) data.

39. The method of claim 7 wherein one nucleic acid strand of the duplex formed in step (a) contains a sequence corresponding to the targeted single nucleotide polymorphism; and the measured amount of initial duplex formed after addition of the target strand indicative of the target strand containing the single nucleotide polymorphism in step (f) decreases as compared to the amount measured in step (b).

40. The method of claim 7 wherein one nucleic acid strand of the duplex formed in step (a) is a wild type sequence; and the measured amount of initial duplex formed after addition of the

target strand is indicative of the target strand containing the single nucleotide polymorphism in step (f) is approximately equal to the amount measured in step (b).

41. The method of claim 8 wherein one nucleic acid strand of the duplex formed in step (a) contains a sequence corresponding to the targeted single nucleotide polymorphism; and the measured amount of initial duplex formed after addition of the target strand indicative of the target strand containing the single nucleotide polymorphism in step (f) decreases as compared to the amount measured in step (b).

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42. The method of claim 8 wherein one nucleic acid strand of the duplex formed in step (a) is a wild type sequence; and the measured amount of initial duplex formed after addition of the target strand is indicative of the target strand containing the single nucleotide polymorphism in step (f) is approximately equal to the amount measured in step (b).

43. The method of claim 9 wherein one nucleic acid strand of the duplex formed in step (a) contains a sequence corresponding to the targeted single nucleotide polymorphism; and the measured amount of initial duplex formed after addition of the target strand indicative of the target strand containing the single nucleotide polymorphism in step (f) decreases as compared

to the amount measured in step (b).

44. The method of claim 9 wherein one nucleic acid strand of the duplex formed in step (a) is a wild type sequence; and the measured amount of initial duplex formed after addition of the target strand is indicative of the target strand containing the single nucleotide polymorphism in step (f) is approximately equal to the amount measured in step (b).

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45. The method of claim 13 wherein the conditions in step (c) comprise heating the titrated solution to a temperature high enough to disrupt the initial nucleic acid duplex and any duplex or triplex formed between the target strand and the first or second nucleic acid strand of the initial nucleic acid duplex, but which do not disrupt the target strand when double-stranded and the conditions of step (d) comprise cooling the titrated solution to a temperature wherein duplex formation occurs.

REMARKS

Claims 1-37 are pending in the instant application. Claims 1-37 have been rejected. Claims 1-15, 25, 28, 30, 31, and 32 have been amended. New claims 38-45 have been added in light of the amendments to claims 1-15, 25, 28, 30, 31 and 32. No new matter has been added by these amendments. Reconsideration is